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Article's main points: We report epidemiological and clinical investigations conducted in France after the worldwide outbreak of *Mycobacterium chimaera* in cardiac surgery. Whole genome sequencing of sequential clinical and HCU isolates helped in classifying the cases and suggest fitness emergence.

Abstract

Background: Post-surgical infections due to *Mycobacterium chimaera* appeared as a novel nosocomial threat in 2015, with a worldwide outbreak due to contaminated heater-cooler units used in open chest surgery. We report the results of investigations conducted in France including whole genome sequencing comparison of patient and HCU isolates.

Methods: We sought *M. chimaera* infection cases from 2010 onwards through national epidemiological investigations in healthcare facilities performing cardiopulmonary bypass together with a survey on good practices and systematic heater-cooler unit microbial analyses. Clinical and HCU isolates were subjected to whole genome sequencing analyzed with regards to the reference outbreak strain Zuerich-1.

Results: Only two clinical cases were shown to be related to the outbreak, although 23% (41/175) heater-cooler units were declared positive for *M. avium* complex. Specific measures to prevent infection were applied in 89% (50/56) healthcare facilities although only 14% (8/56) of them followed the manufacturer maintenance recommendations. Whole genome sequencing comparison showed that the clinical isolates and 72% (26/36) of heater-cooler unit isolates belonged to the epidemic cluster. Within clinical isolates, 5 to 9 non-synonymous single nucleotide polymorphisms were observed, among which an *in vivo* mutation in a putative efflux pump gene observed in a clinical isolate obtained for one patient under antimicrobial treatment.

Conclusions: Cases of post-surgical *M. chimaera* infections were declared to be rare in France, although heater-cooler units were contaminated as in other countries. Genomic analyses confirmed the connection to the outbreak and identified specific single nucleotide polymorphisms, including one suggesting fitness evolution *in vivo*.

Keywords: heater-cooler units (HCU), mmpL, nontuberculous mycobacteria (NTM), molecular epidemiology

Introduction

Since the Rapid Risk Assessment (RRA) alert issued by the European Centre for Disease Prevention and Control (ECDC) in 2015 (1), over 100 cases of invasive cardiovascular infection and disseminated disease with *Mycobacterium chimaera* have been reported worldwide, not only in Europe, but also in North America and Asia (2,3). These infections are difficult to diagnose because of nonspecific symptoms and specific microbiological requirements (4). These infections were attributed to contamination from the heater-cooler units (HCU) present in operating rooms since *M. chimaera* was found in their water tanks, probably transmitted on the surgical field through aerosolization when the exhaust fans were running (5). Genomic studies on *M. chimaera* isolates showed, on the one hand, that most of the clinical isolates collected in different countries were clustered, and in the other hand, that many isolates cultured from the HCU water samples belong also to this cluster (6–8). This raised the hypothesis of a common reservoir at the HCU factory. However, since these infections occurred over many years, mean incubation being 21 months (2), and as *M. chimaera* is often present in water networks (9), all nosocomial infections might not be attributed to the factory contamination.

M. chimaera is a slow growing nontuberculous mycobacterium (NTM) belonging to the *Mycobacterium avium* complex (MAC), now described as a subspecies of *M. intracellulare* (10). It was rarely recognized as a pathogen before the outbreak and rarely identified at the species or subspecies level (11). The purpose of the present study was to report the findings of the epidemiological, microbiological surveillance and molecular investigations conducted with Health care facilities (HCF) practicing open-chest surgery in France. Clinical isolates

were characterized for single nucleotide polymorphisms (SNP) observed in comparison to HCU isolates. We also investigated the *in vivo* mutations that emerged between sequential isolates from the same patient.

Methods

Epidemiological investigations

Two investigations were organized targeting the 61 HCF where cardiothoracic surgery was regularly performed under cardiopulmonary bypass (CPB) in France. The first, performed immediately after the ECDC June 2015 alert, sought clinical cases that could have occurred from January 1st, 2010 to April 30th, 2015 (12). The second investigation, performed in 2017, sought additional cases from 2015 onwards and questioned about HCU good practices including HCU microbiological analysis (13). These investigations are detailed in the Supplemental Material and in Supplemental Table 1 and 2.

M. chimaera detection in water tank samples of heater-cooler units

A protocol adequate for water network samples was adapted from Radomski *et al.* (14), based on filtration and culture on specific media for mycobacteria research as detailed in the Supplemental Material. Species and subspecies identification among the *Mycobacterium avium* complex was done using GenoType NTM-DR 1.0 (Bruker, Nehren, Germany) and ITS or *hsp65* PCR-sequencing when necessary (11).

Whole genome sequencing and SNP analysis

DNA was extracted from *M. chimaera* solid cultures on Middlebrook 7H10 agar using DNA Ultraclean Microbial kit (QIAGEN, Hilden, Germany). DNA libraries were prepared with Nextera XT kit (Illumina, San Diego, USA), and whole genome sequencing (WGS) was performed with a MiSeq system (Illumina) and the MiSeq Reagent V2 (2x150) kit (Illumina). Sequence reads were aligned to the reference genome of *M. chimaera* strain ZUERICH-1 (sequence ID NZ_CP015272.1 (7)) using Bionumerics version 7.6 (Applied Maths, Gent, Belgium). The reads were trimmed excluding base call with a Phred score below 15. The SNP signature was built using the strict-filtering (closed SNP set) option, retaining SNP present in all the isolates, with a minimum coverage of 5X covered at least once in both forward and reverse directions, a minimum distance between the retained SNP position of 12 base pairs and removing non-discriminatory position. The SNP matrix was used to build a maximum parsimony tree. Isolates were classified using the allele typing proposed by van Ingen *et al.* (7). Genomic sequences are available from the GenBank/NCBI in BioProject PRJNA667507.

Results

***M. chimaera* clinical cases**

Two cases (Patients P1 and P2) of disseminated *M. chimaera* disease were related to the outbreak out of four suspected cases reported by HCF as the first investigation results. The two excluded cases were a patient with *M. chimaera* cultured from a pericardial fluid but not having been submitted to CPB, and a patient with a post-CPB infection but the isolate was *M. avium* and not *M. chimaera*. P1 was a 61-year-old man, who underwent cardiac surgery to replace part of his ascending aorta with a bio-prosthetic graft in 2012. He

developed osteo-articular infection in 2014 (isolate P1a, Table 1) and bloodstream infection was still diagnosed one year after (isolate P1b) (2,12). He died 6 years later although being treated by a 4-antibiotic regimen, combining azithromycin, ethambutol, rifampicin, and moxifloxacin. P2 was a 53-year-old man who was diagnosed with disseminated granulomatous disease a few months after cardiac surgery involving a graft replacement of the aortic valve for aorta dilatation in 2009 and 2010 (12). The patient presented *M. chimaera*-positive blood cultures two years after the surgery and died shortly after. No invasive *M. chimaera* infection cases were reported after 2015.

Contamination of HCU water samples with *M. chimaera* and HCU practice maintenance

56 HCFs (92% of all HCF) answered to the questionnaires, reporting the use of 227 HCUs. Specific measures to prevent infection were applied in 89% of them (50/56). Only 14% (8/56) systematically followed the manufacturer maintenance recommendations and this dropped to 7% (4/56) for the new measures issued by Livanova in 2015, such as an increase in the frequency of water replacement and disinfection of the water circuit, see Supplemental Table 2.

HCFs declared that 41 (23%) of the 175 HCU cultivated for MAC were positive. Our national reference laboratory received 75 additional HCU water samples from 11 HCFs, out of which 36 (48%) were positive for various species of mycobacteria, see Supplemental Table 3 and Supplemental Figure 1. Out of these 36 positive HCU samples, 24 were identified positive with *M. chimaera* at a mean quantification of 10^4 CFU/L (range 10^1-10^5/L). HCUs were also positive with some other mycobacteria: 17 with *M. chelonae* (mean 3.3 x 10^3 CFU/L), 8 with *M. mucogenicum* (mean 1.1 x 10^3 CFU/L), 8 with *M. gordonae/paragordonae* (mean 3.4 x

10^3 CFU/L), 2 with *M. avium* (mean 8 x 10^3 CFU/L) and 2 with *M. peregrinum* (mean 1.2 x 10^3 CFU/L).

Genomic comparison of *M. chimaera* isolates

A total of 57 *M. chimaera* isolates (detailed in Figure 1 and Supplemental Table 4) were submitted to WGS analysis: the three clinical isolates obtained from the two patients (P1a, P1b, P2), 36 isolates from HCU water samples of 11 HCF, 17 clinical *M. chimaera* isolates *a priori* unrelated to the outbreak (from two myocardial and one breast biopsies, and 14 sputum), and one environmental isolate from the water supply network of a French hospital. WGS data were compared to the genome sequence of the epidemic strain *M. chimaera* ZUERICH-1 (7). Sixteen other genomes published for clinical cases described in the USA (*n* = 13 (15) and in Ireland (*n* = 3) were added to the comparison (16).

Genomic comparison analysis generated 55,638 SNPs, which were used to build a maximum parsimony tree, shown in Figure 2a. The genomes distributed among 9 groups, with 52 isolates clustering with the epidemic strain *M. chimaera* ZUERICH-1, namely group 1, in which less than 100 SNP differences were observed between the sequences (7). A subsequent analysis of these 52 genomes generated 694 SNPs, which were used to build a second maximum parsimony tree, shown in Figure 2b. This showed more precisely 30 isolates clustering with the epidemic strain *M. chimaera* ZUERICH-1, with a mean pairwise distance of 4 SNP. The three isolates corresponding to the French clinical cases (P1 and P2 isolates) were confirmed to belong to the epidemic cluster as well as 26 out of 36 (72%) *M. chimaera* isolates isolated from HCUs used in French hospitals. All were Livanova® HCU and none from Maquet® ones. Clinical isolates were typed according to allele classification proposed by van Ingen *et al.* and belong to 1.1 subgroup as ZUERICH-1 (7), see

Supplemental Table 4. One isolate (#8) obtained from a sputum sample of a non-related patient with no history of cardiac surgery, was unexpectedly phylogenetically linked to the epidemic cluster but with 16 SNP differences and belonged to subgroup 1.8.

Specific analyses of clinical isolates

We specifically analyzed the genomes of the three clinical isolates from the two cases, detailing the SNP observed with regards to the epidemic strain Zuerich-1, as presented in Table 2. Among 11 SNPs, all located in a protein coding sequence and only one was synonymous. Minimum inhibitory concentrations (MIC) of several antimicrobial agents were determined for the clinical isolates and are shown in Table 1.

Between the two sequential isolates from patient P1, P1a isolates in 2014 and P1b isolates in 2015, 5 SNPs were observed. One SNP produced a STOP codon in the gene *MYCOZU1_RS21880*. This gene contains a conserved domain belonging to the mycobacterial membrane protein large (mmpL) family, a resistance-nodulation-cell division (RND) family transporter reported to be involved in multi-substrate transport, and contribute to virulence and pathogenicity (17). *MYCOZU1_RS21880* exhibits a 67.8% similarity at the nucleotide level with *M. tuberculosis mmpL5*, see Supplemental Table 5. However, the *M. chimaera mmpL5* ortholog would more likely correspond to the gene *MYCOZU1_RS13900*, which presents a higher similarity (76.2%) and is found in synteny with an *mmpS*-like gene (*MYCOZU1_RS13905)* and a *tet-R*-like gene (*MYCOZU1_RS13910)* upstream (18). The SNP was observed in the genome of the P1b isolate, which was isolated after 1 year of antibiotic treatment including azithromycin, ethambutol, rifampicin, and moxifloxacin.

No HCU isolates were obtained from the hospital where P1 was operated on, since the samples were declared negative for many months after the case was declared. P2 was

operated on in hospital H5 and diagnosed in hospital H14. Fourteen water samples were recovered from this hospital and 9/14 (64%) were positive with *M. chimaera*. In total, 10 HCU isolates were sequenced. Genomic comparison of P2-related isolates (clinical and environmental) is shown in Figure 3. Five HCU isolates showed more than 100 SNPs difference and thus were not related to the outbreak. Five HCU isolates belonged to the epidemic cluster, showing 1 to 14 SNP differences from the P2 isolate sequence, which are presented in Table 2. Half of those SNPs were synonymous.

Discussion

Contamination of HCU water cooling systems by *M. chimaera* has led to a worldwide outbreak among patients undergoing cardiopulmonary bypass surgery (2). In this study, we report the results of investigations and surveillance conducted in France, focusing on the results of genomic analyses of *M. chimaera* isolates obtained from patients and HCU contamination related to the outbreak. Although most of the isolates clustered with the outbreak strain Zuerich-1, some did not, and within the cluster several SNP differences resulting in substitutions in gene coding regions were observed.

In the search for clinical cases related to the outbreak in all the medical facilities performing open-chest surgery with cardiopulmonary bypass in France, only two cases were declared, which is much less than in other European countries (e.g. 18 cases in the United Kingdom (6), six cases in Switzerland (19)). This could be due to a shorter period of investigation (5 years versus 8 or 9 years respectively) or differences in practices. Another explanation could be under-reporting or under-diagnosis of cases since not all HCFs are searching for *M. chimaera* infections in re-operated patients in infectious contexts (10% of cases in France).

However, cases were also sought through an active network of cardiologists, surgeons and microbiologists which regularly surveyed infective endocarditis without further new cases detected (20). The majority of HCFs declared, however, having implemented prevention measures and maintenance protocols to reduce exposure of patients to HCUs, so that even if compliance to manufacturer recommendations was not complete or loosely adapted to the reality of the practice, this could explain the small number of cases despite the positivity of many HCU water samples.

The manufacturers revised their recommendations after the ECDC alert and they were difficult to apply. For example, the daily exchange of the total water volume from the reservoir was not possible due to the architecture of the water tank and it requires too much personal time. The HCF personal and staff however performed disinfection with the elements and materials they had and as frequently as possible. It was eventually shown that the HCUs were mainly contaminated during their production at the factory and consequently, the disinfection measures recommended were mainly useful to decrease the *M. chimaera* load but not prevent its contamination of the water contained in the HCUs (4).

Following a protocol standardized for mycobacterial detection and applied to HCU water samples, we showed that *M. chimaera* was the most frequently isolated and abundant mycobacteria. MAC are waterborne, and *M. chimaera* can be recovered from various environment: drinking water distribution systems, household water, and associated biofilms (9). MAC can be found up to 10^6 UFC/L in water distribution systems (21). Strikingly, slow-growing mycobacteria were more prevalent in HCU water than rapid-growing mycobacteria, contrary to what is observed in the water-supply network in France (22). Other mycobacterial species were also detected in HCU water (23) and their presence in the

operating room during cardiac surgery is a potential nosocomial threat. Infection with *M. abscessus* (24) and *M. wolinskyi* (25) linked to contaminated HCUs were indeed previously reported, and *M. chelonae* was one of the mycobacterial species most frequently found in endocarditis (26). *M. chimaera* HCU contamination could also put healthcare workers at risk of developing pneumonia (27).

WGS and SNP analysis has become the reference method for the molecular epidemiological study of *M. tuberculosis* (28), but few studies on NTM are available. WGS was used to connect *M. avium* isolated in household water to respiratory disease, showing a maximum distance of 51 SNP between respiratory and household isolates (9). Concerning the *M. chimaera* outbreak, several WGS studies compared clinical and HCU isolates and showed a clonal signature with a mean pairwise distance ranging from 4 to 10 SNP differences (6–8). Using the same methodology, we found very low diversity in the epidemic cluster for the *M. chimaera* isolates in France, with a mean pairwise distance of 4 SNP and confirmed that the two suspected patients were infected by the *M. chimaera* epidemic strain. In our study, 72% of *M. chimaera* found in HCU water samples belonged to the epidemic group cluster. Other studies also found a predominance of the epidemic clone, with levels ranging from 48% (6) to 96% of the studied isolates (8). Surprisingly, one isolate not related to the outbreak was shown to belong to the epidemic cluster. This was also observed in another study in the UK that identified two isolates from control patients as belonging to the epidemic cluster (6). Bioinformatic analysis is a major source of analysis variation that can affect SNP variant calling (28). WGS analysis is a powerful, discriminative tool, but we do not know its exact specificity.

Our study has several limits. Case research and HCU surveillance were not systematic but based on declaration and retrospective research, as the mycobacteriology laboratories are not centralized like in UK, for instance (29). All the HCU isolates were sampled after 2015, although contamination occurred a few years before. There were indeed several years delay between patient contamination and microbial analysis of HCU water samples (6–8). Lastly, since we decontaminated the water sample, we cannot know about contamination by bacteria other than mycobacteria, as was reported by M. Chand *et al.* (6).

Specific SNP analysis of clinical and HCU isolates revealed diversity, even for those included in the epidemic cluster. Interestingly, SNP observed in the clinical isolates showed very few non-synonymous mutations, compared to SNP observed in the HCU isolates. This could suggest that only SNP conferring adaptive advantage (survival, virulence) are selected during in-host evolution. Experimental studies have shown that only a few mutations can increase virulence. In a follow-up study on *M. avium* respiratory diseases, the genome sequences of intra-patient isolates were highly similar, with only 0 to 19 SNP differences, but exhibited increased virulence features compared to the first isolates (30). Patient P1b's isolate showed a nonsense mutation in a MmpL protein that was among the additional *mmpL* genes found in *M. chimaera* but not *M. tuberculosis* (31) and exhibited homology with *mmpL5*. MmpL5 protein is an RND superfamily efflux pump involved in siderophores and the export of drugs such as azole and bedaquiline (18). The P1b *mmpL5* mutated isolate showed decreased MIC value compared to the parent strain P1 isolated one year before. In *M. tuberculosis*, *mmpL5* was shown to be a non-essential gene in the presence of heme and haemoglobin as iron source (32). Further studies are required to determine if this mutation is linked to an increase in virulence or fitness.

Conclusion

In conclusion, we have presented a complete epidemiological investigation of *M. chimaera* infection associated with heater-cooler units in France through practice assessment, microbiological surveillance, case detection, and molecular analysis. Two cases were reported and confirmed by whole genome analysis. Due to the high prevalence of the *M. chimaera* epidemic strain found in HCUs, regulatory action and continuous surveillance are still necessary.

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Potential Conflicts of Interest

The authors report no conflict of interest.

Patient Consent Statement

Written informed consent was obtained from patient's next of kin.

We followed the procedures in accordance with the Helsinki Declaration. Information were anonymized. According to the French regulation on observational database analyses including microbial database, the study did not need specific ethical requirements.

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Table 1. Main characteristics of the *M. chimaera* clinical isolates

Characteristic	Patient 1		Patient 2
Isolate*	P1a (I58)	P1b (I57)	P2 (I18)
Hospital*	H25	H25	H14
SNP difference with strain Zuerich-1	5	10	5
Clarithromycin MIC	2	0.5	2
Rifabutin MIC	2	0.25	0,5
Ethambutol MIC	16	4	16
Isoniazid MIC	> 8	2	2
Moxifloxacin MIC	4	2	4
Rifampicin MIC	> 8	2	4
Sulfamethoxazole/trimethoprim MIC	8/152	1/19	8/152
Amikacin MIC	8	4	16
Linezolid MIC	32	4	16
Ciprofloxacin MIC	16	8	16
Streptomycin MIC	16	8	32
Doxycycline MIC	16	>16	>16
Ethionamide MIC	20	1.2	10
Bedaquiline MIC	0.25	0.25	0.25

*See details in the Supplemental Table 4 list of isolates

MIC: minimal inhibitory concentration in mg/L, determined using the commercial microdilution method, SLOMYCO Myco Sensititre™ (Thermo Scientific™) following the manufacturer recommendations and CLSI guidance for bedaquiline testing (Woods GL, et al. Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes [Internet]. 2nd ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2011 Mar. Report No.: M24-A2. PMID: 31339680.).

Table 2. SNP analyses determined from the genomic comparison of clinical isolates from patients P1 and P2 and of environmental isolates related to the P2 case

Isolates	Position[1]	Nucleotide modification	Gene characterization	Codon	Mutation effect
Comparison of clinical isolates from the P1 and P2 cases					
P1a, P1b, P2	2 587 843	T -> C	glycosyltransferase	CAC->CGC	H->R
P1a, P1b, P2	3 709 626	G -> A	type III polyketide synthase	GCG->GTG	A->V
P1a, P1b, P2	4 520 250	T -> A	signal peptidase I	GAA->GAT	E->D
P1a, P1b, P2	4 919 479	A -> G	helix-turn-helix transcriptional regulator	TCC->CCC	S->P
P1a, P1b	2 67 758	C -> A	hypothetical protein	GAC->TAC	D->Y
P1b	2 22 733	T -> C	decaprenyl-phosphate phosphoribosyltransferase	TTC->CTC	synonymous
P1b	1 256 515	G -> T	IS481 family transposase	AGC->ATC	S->I
P1b	1 256 515	G -> T	NAD(P)-dependent oxidoreductase	AGC->AGA	S->R
P1b	1 509 107	C -> G	LuxR family transcriptional regulator	CGC->GGC	R->G
P1b	4 708 684	G -> A	RND family transporter (mmpL5)	TGG->TGA	**W->UGA**
P1b	5 517 224	T -> A	porphobilinogen synthase	ATG->TTG	M->L

P2	2 436 71	T -> C	arabinosyltransferase	ATG->GTG	M->V
Comparison of environmental isolates related to the P2 case					
I36, I35	5 234 848	A -> G	FAD-dependent oxidoreductase	CAC->CGC	H->R
I36, I35	5 947 828	C -> A	NAD(P)/FAD-dependent oxidoreductase	GAC->AAC	D->K
I35	3 350 727	A -> T	PPE family protein	CTG->CAG	L->Q
I30, I34	1 367 077	T -> C	hypothetical protein	GTG->GCG	V->A
I30, I34	2 057 124	C -> A	valine-tRNA ligase	CCC->CCA	synonymous
I30, I34	5 952 067	G -> C	cytochrome P450	CAC->CAG	H->Q
I30, I34	5 952 114	G -> T	NIPSNAP family protein	CTC->CTA	synonymous
I30, I34	6 159 327	G -> A	hypothetical protein	CGC->CAC	R->H
I30	539 741	C -> A	SpoIIE family protein phosphatase	CGT->CTG	R->L
I30	576 340	C -> T	type II secretion system F family protein	CTG->TAG	L->STOP
I30	4 243 378	C -> G	hypothetical protein	GGG->GGC	synonymous
I34	1 204 266	G -> T	LuxR family transcriptional regulator	GCC->GCA	synonymous
I34	1 562 307	G -> A	alpha/beta hydrolase	CAG->CAA	synonymous
I34	2 093 313	C -> T	PE-PPE domain-containing protein	GCG->GCA	synonymous
I34	2 447 021	T -> C	DNA primase	ATC>ACC	I->T
I34	2 842 316	C -> A	MCE family protein	CTG>CTT	synonymous

I34	4 179 540	T -> C	hypothetical protein	ACA>ACG	synonymous
I34	4 243 378	C -> G	hypothetical protein	GGG>GGC	synonymous
I34	5 019 553	G -> A	unknown function	CCC>CCT	synonymous

[1] SNPs were generated with regards to the sequence of the epidemic strain *M. chimaera* ZUERICH-1 (NCBI reference sequence NZ_CP015272.1).
[2] The SNP at 1256515 position affected 2 genes, one forward and the other in reverse.

Figure 1. Overview of studied *Mycobacterium chimaera* isolates and their origin according to the epidemiological investigations

SHCF: health care facility, HCU: heater-cooler unit, MAC: Mycobacterium avium complex, WGS: whole genome sequencing

Figure 2. Phylogenetic analysis of *M. chimaera* isolates

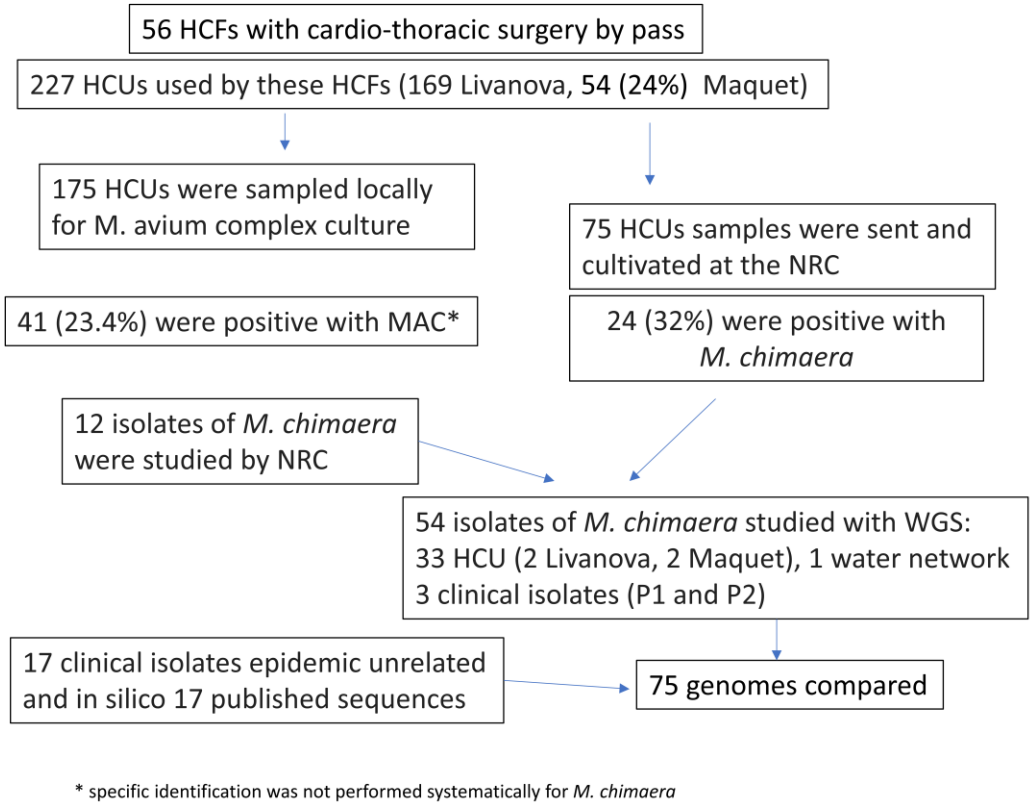
Figure 2A: Maximum parsimony tree (logarithmically scaled) built based on the 55 638 SNP positions found through the comparison of the 75 genomes that were mapped to the reference genome of *M. chimaera* ZUERICH-1. The 52 isolates, including Zuerich-1, belonging to group 1 are gathered in a green circle.

Figure 2B: Maximum parsimony tree (logarithmically scaled) built based on the 694 SNP positions found through the comparison of the genomes that were mapped to the reference genome of *M. chimaera* ZUERICH-1. The number of SNP differences with regard to the epidemic strain *M. chimaera* ZUERICH-1 is indicated inside each circle. Isolates from HCU water samples are labelled in blue, isolates from clinical samples in purple, and isolates from clinical samples related to the outbreak in red. § indicates two isolates isolated from the same patient.

Figure 3: Phylogenetic analysis of *M. chimaera* isolates related to P2

Figure 3: Maximum parsimony tree (logarithmically scaled) built based on the 21 106 SNP positions mapped to the genome of *M. chimaera* ZUERICH-1 found through the comparison of the 11 isolates related to patient P2. The number of SNP differences compared to patient P2's isolate is indicated inside each circle. Isolates from HCU water samples are labelled in blue, and isolates from clinical samples related to the outbreak in red.

Figure 1



* specific identification was not performed systematically for *M. chimaera*

Figure 2A

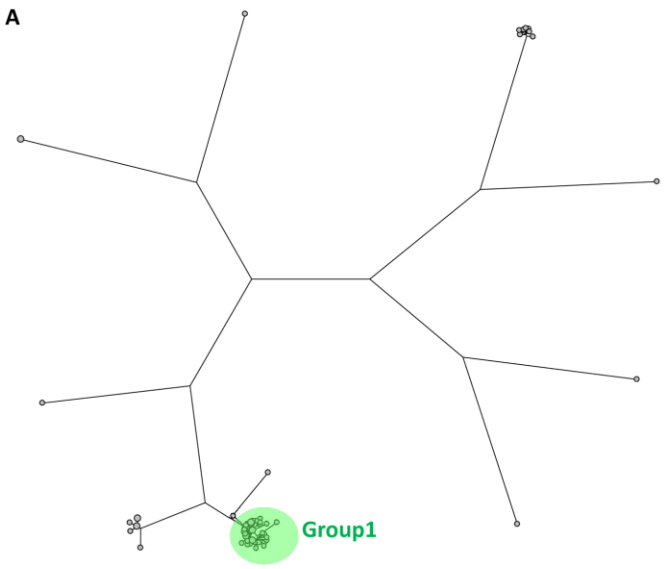


Figure 2b

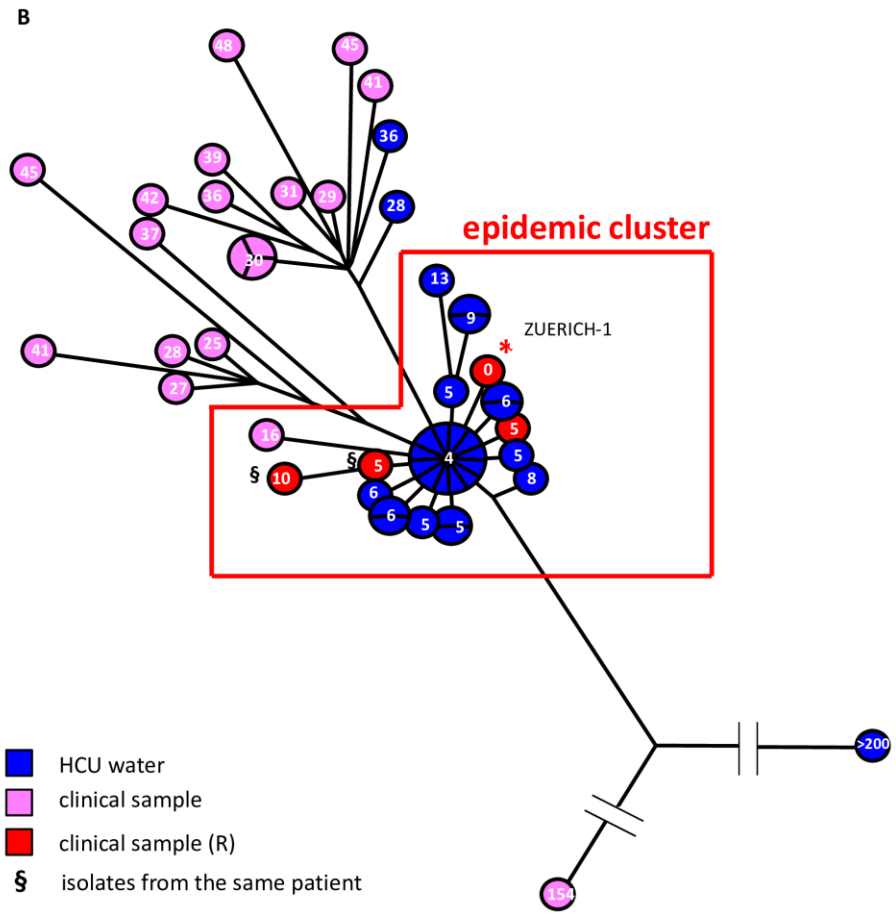


Figure 3